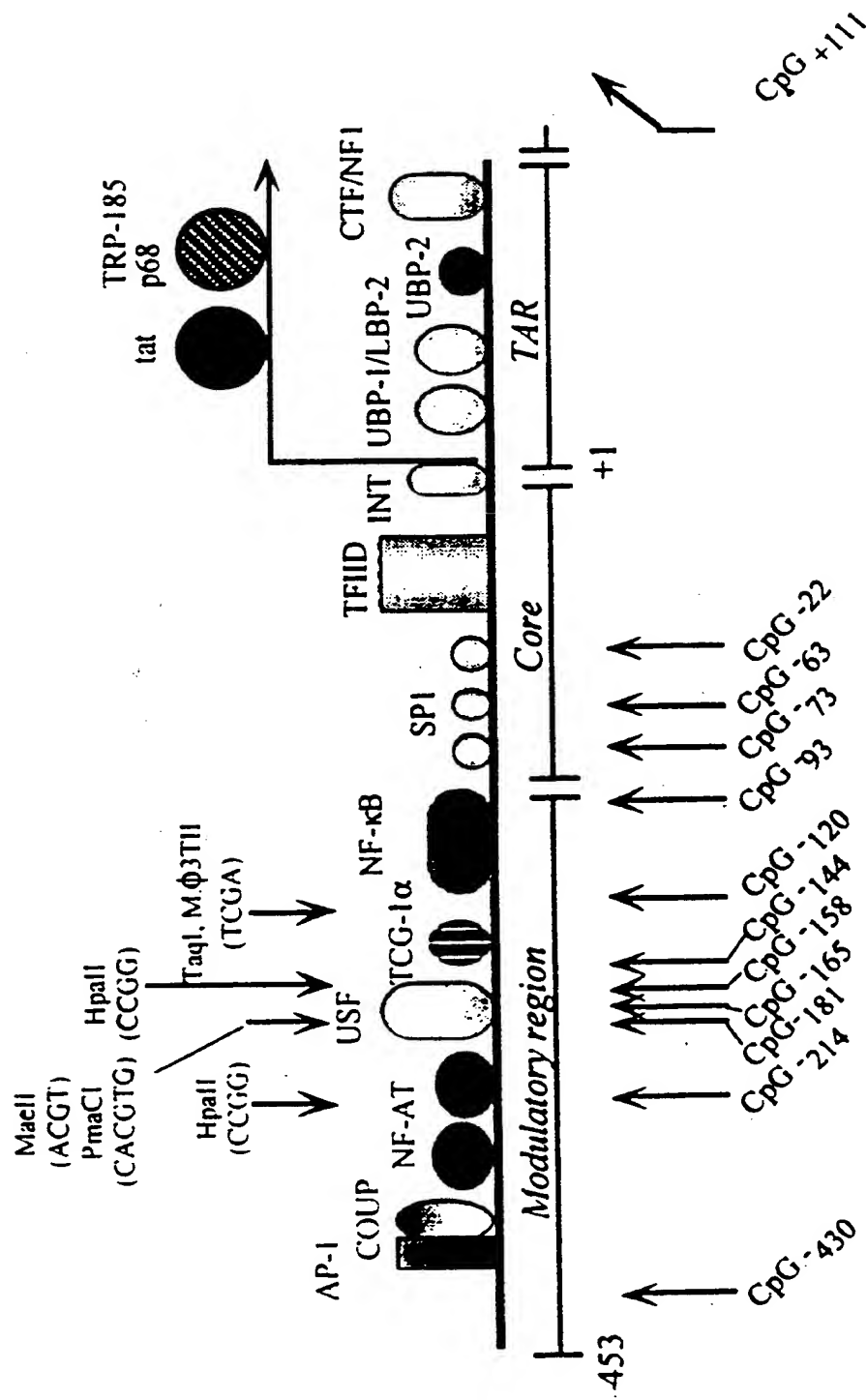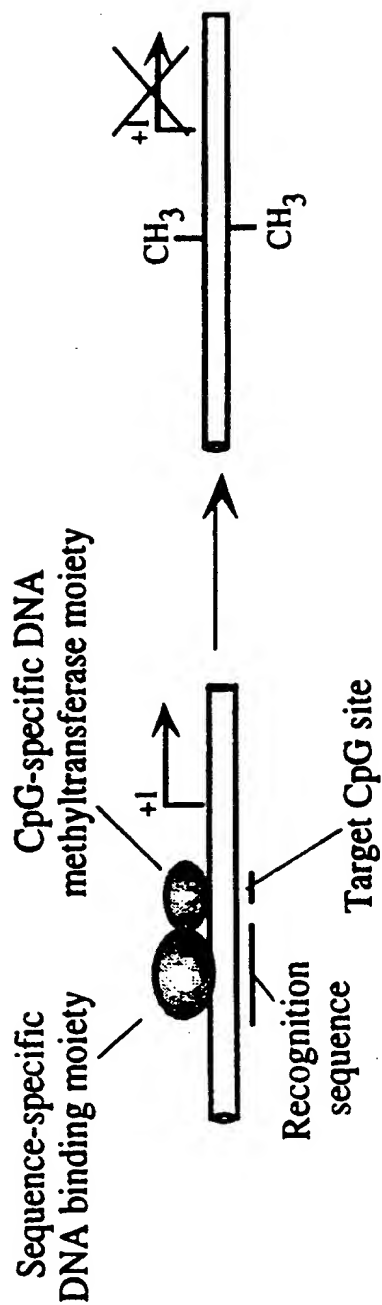## FIGURE 1

FIGURE 2

-454 TGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTC

CCTGATTAGCAGAGAACTACACACCAGGGCCAGGAGTCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCC

AGATAAGGTAGAAGAGGCCAACAAGAGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGGATGATGACCCGGATATAT

AAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCCATTCATCACGTGGCCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCTGA

TATCCGAGCTTGCTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGCGTGGCCTGGGCCGCGGGACTGGGGAGTGGCGAGCCCTCAG

ACCTGCATATAAGCAGCTGCTTTTTGCCTGTACT+1GGGTCTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCCTCTCTGGCTAGCT

AGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACT

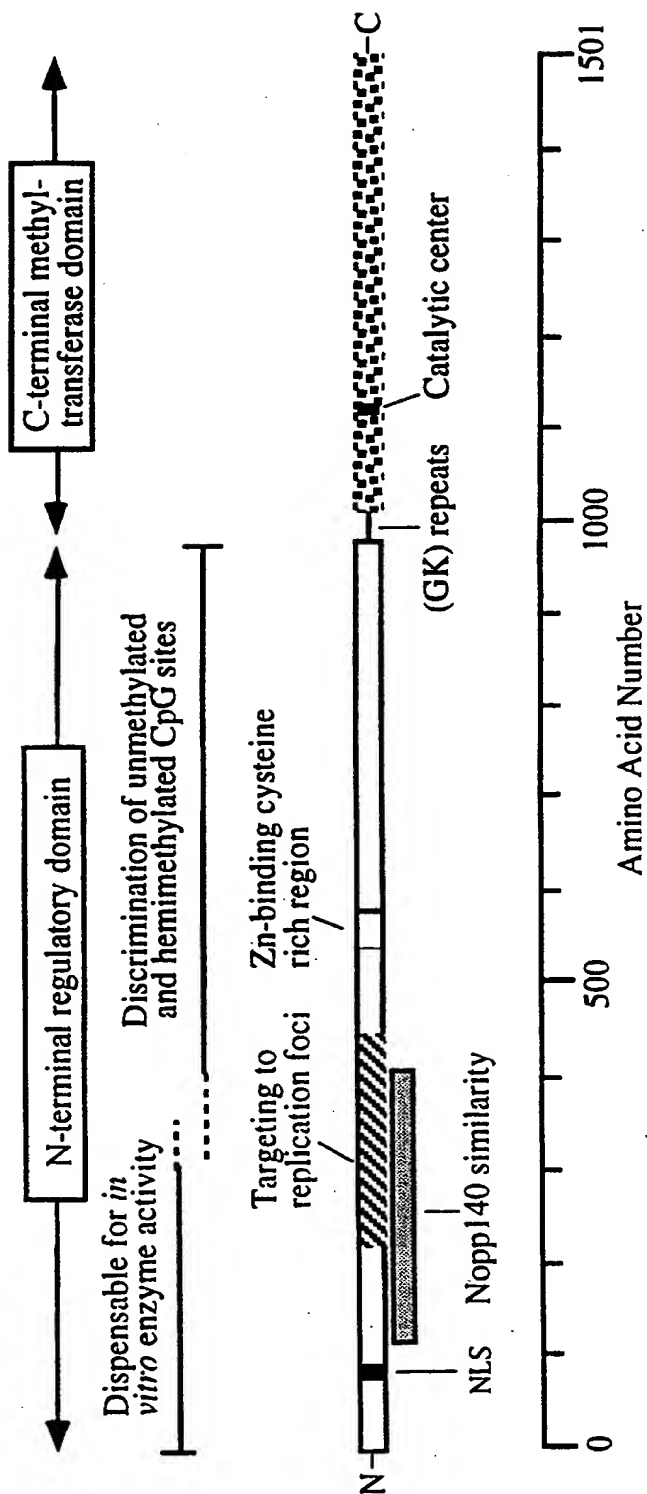AGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGC.. +200

FIGURE 3

Sequence-specific
DNA binding moiety

CpG-specific DNA
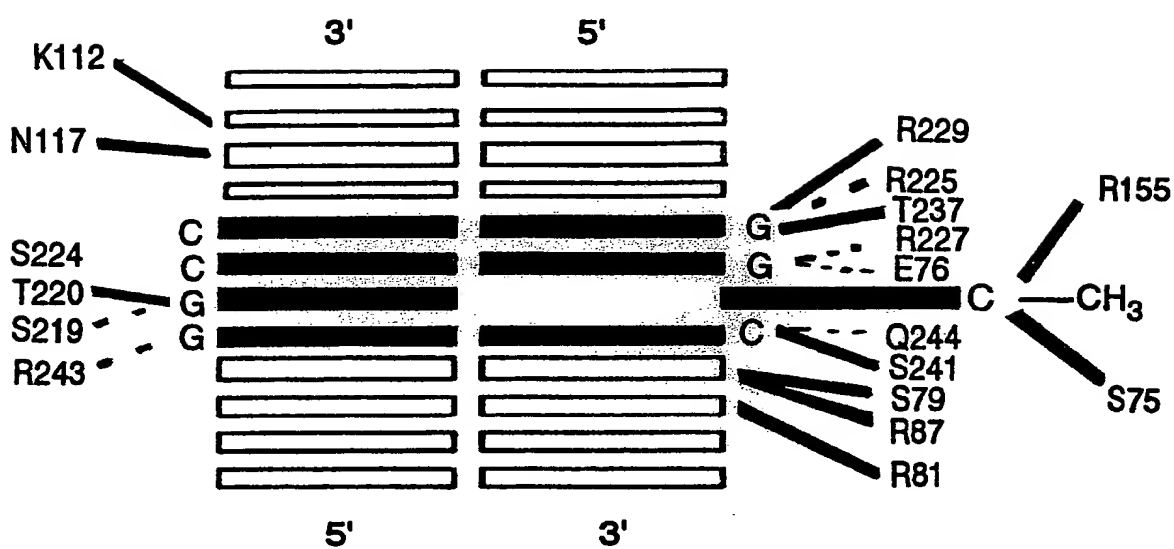methyltransferase moiety

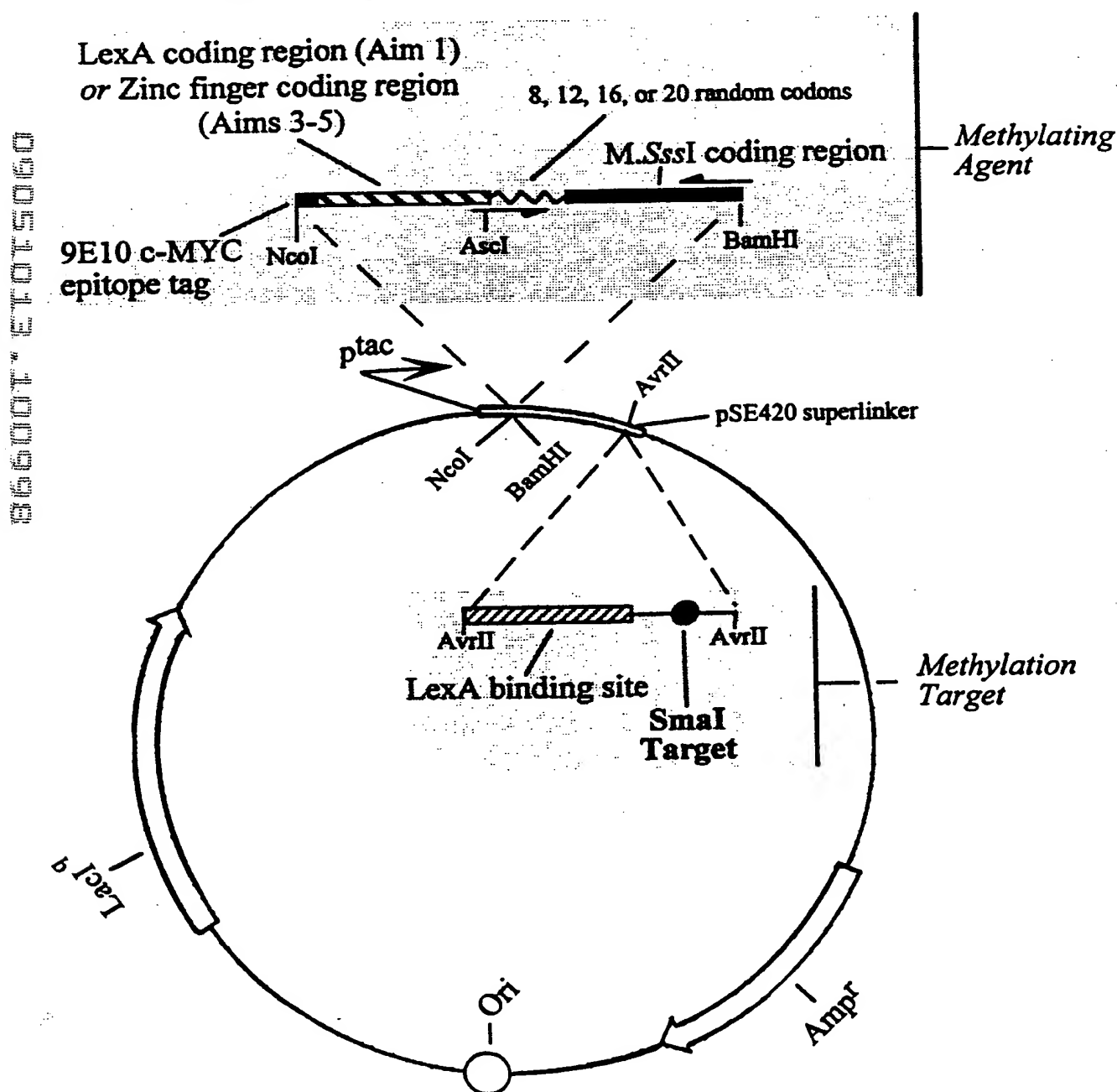Recognition
sequence

Target CpG site

CH₃

CH₃

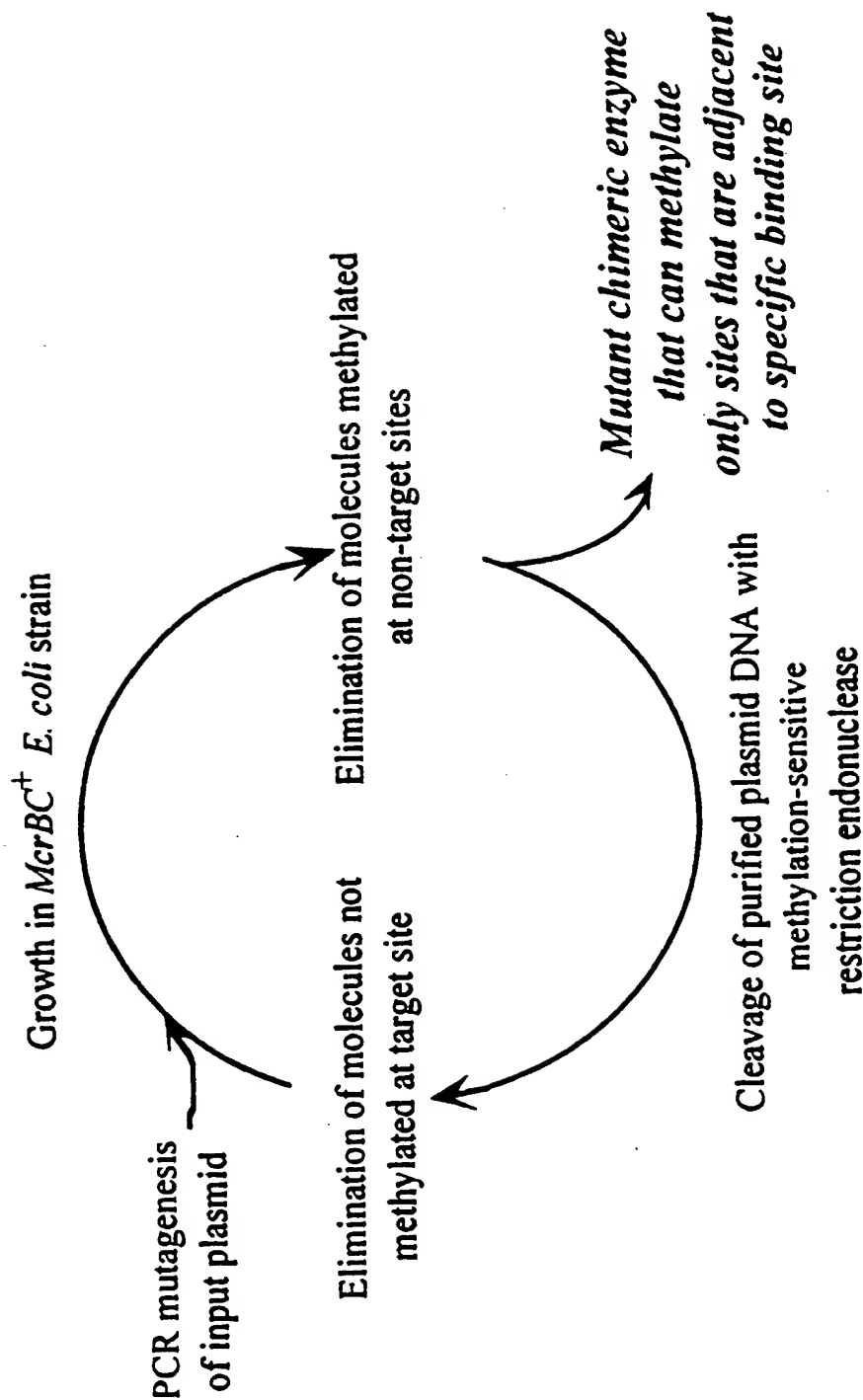## FIGURE 4

FIGURE 5

FIGURE 6

pLS, An Expression Construct to be Used in the Selection of DNA Binding Protein/DNA Methyltransferase Chimeras That Methylate Predetermined Sequences
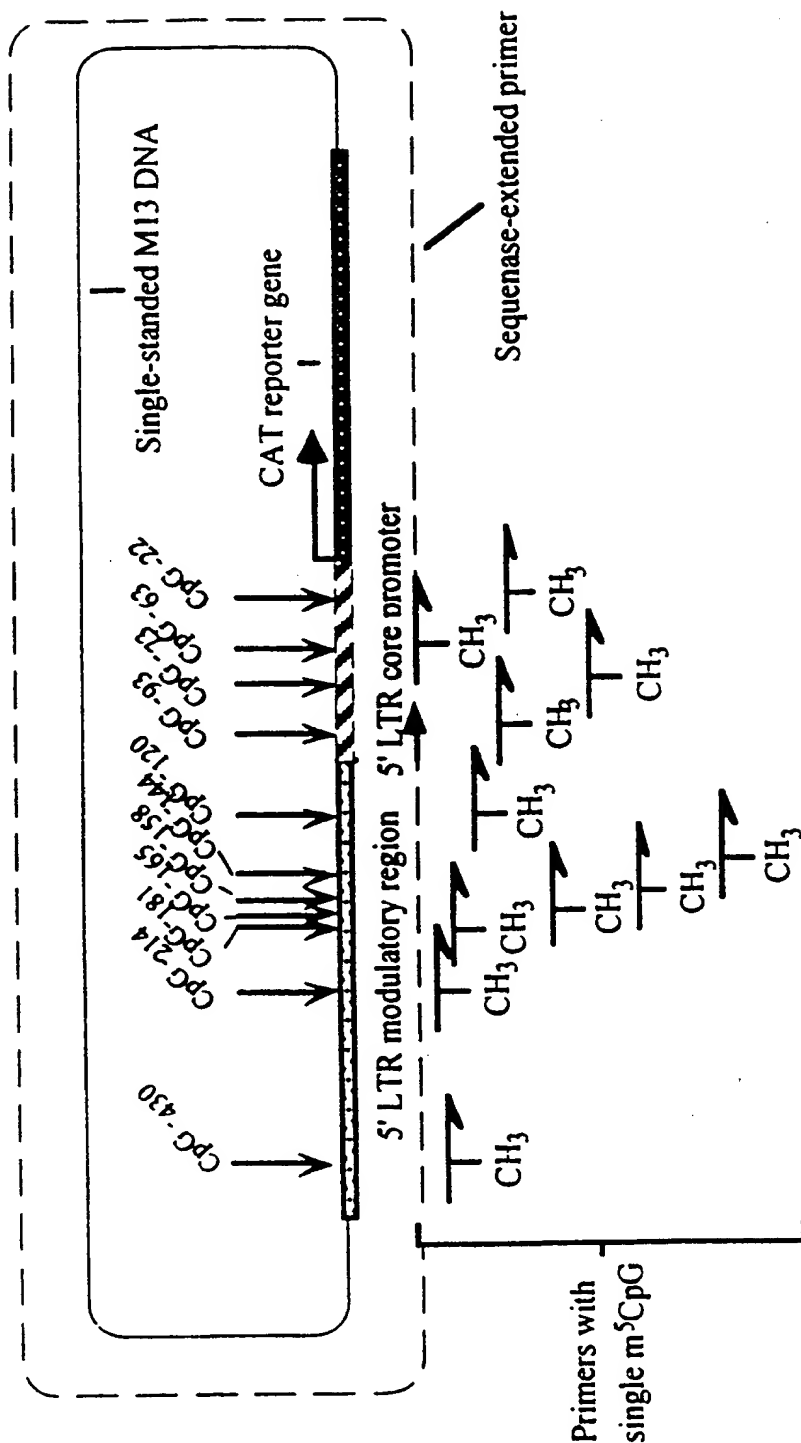
FIGURE 7

Cyclic in vitro/in vivo selection for chimeric DNA binding protein-DNA methyltransferases

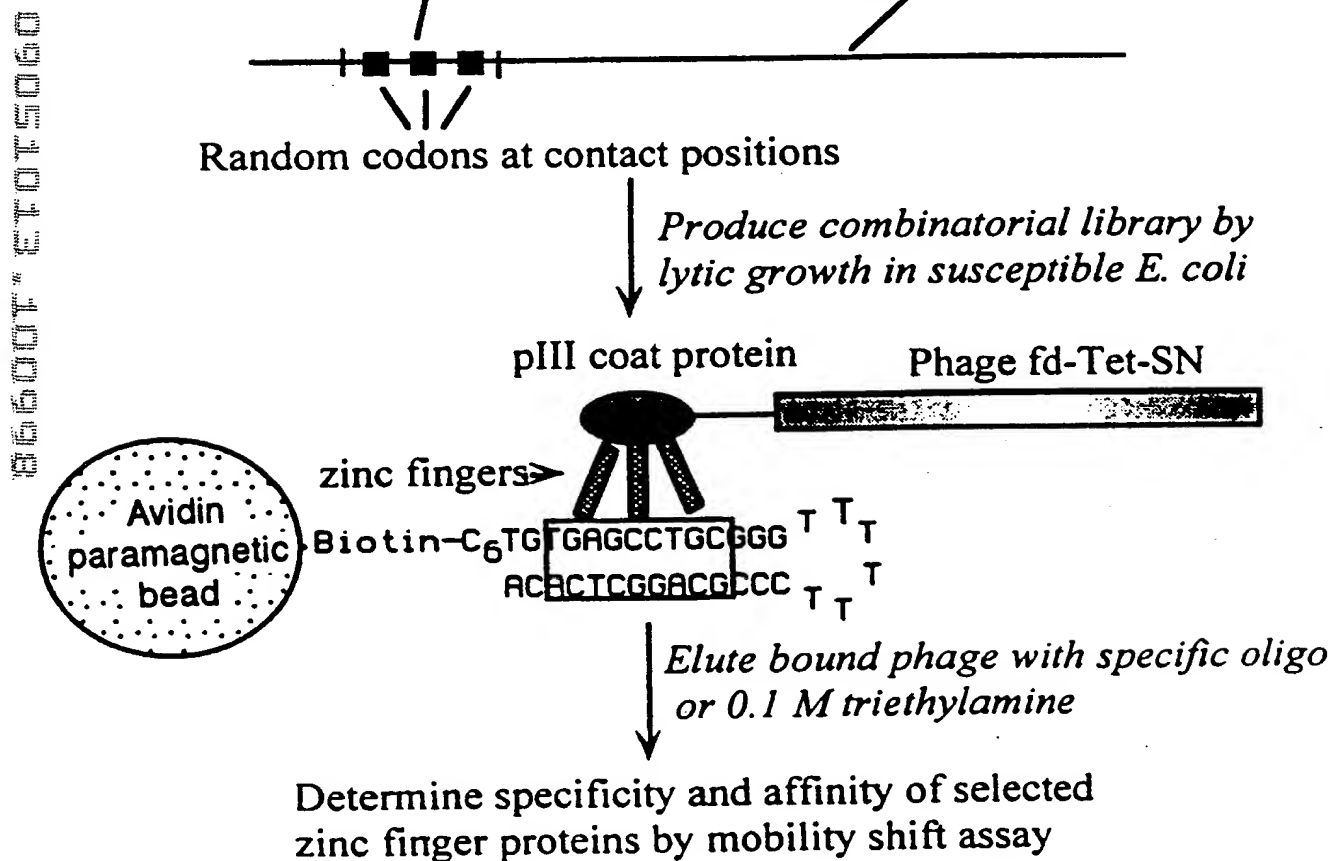Growth in *McrBC*[+] *E. coli* strain

Elimination of molecules methylated at non-target sites

Mutant chimeric enzyme that can methylate only sites that are adjacent to specific binding site

Cleavage of purified plasmid DNA with methylation-sensitive restriction endonuclease

Elimination of molecules not methylated at target site

PCR mutagenesis of input plasmid

FIGURE 8

FIGURE 9

Phage-display selection of Zinc-Finger Proteins that Bind to Predetermined Sequences in the HIV-1 5' LTR

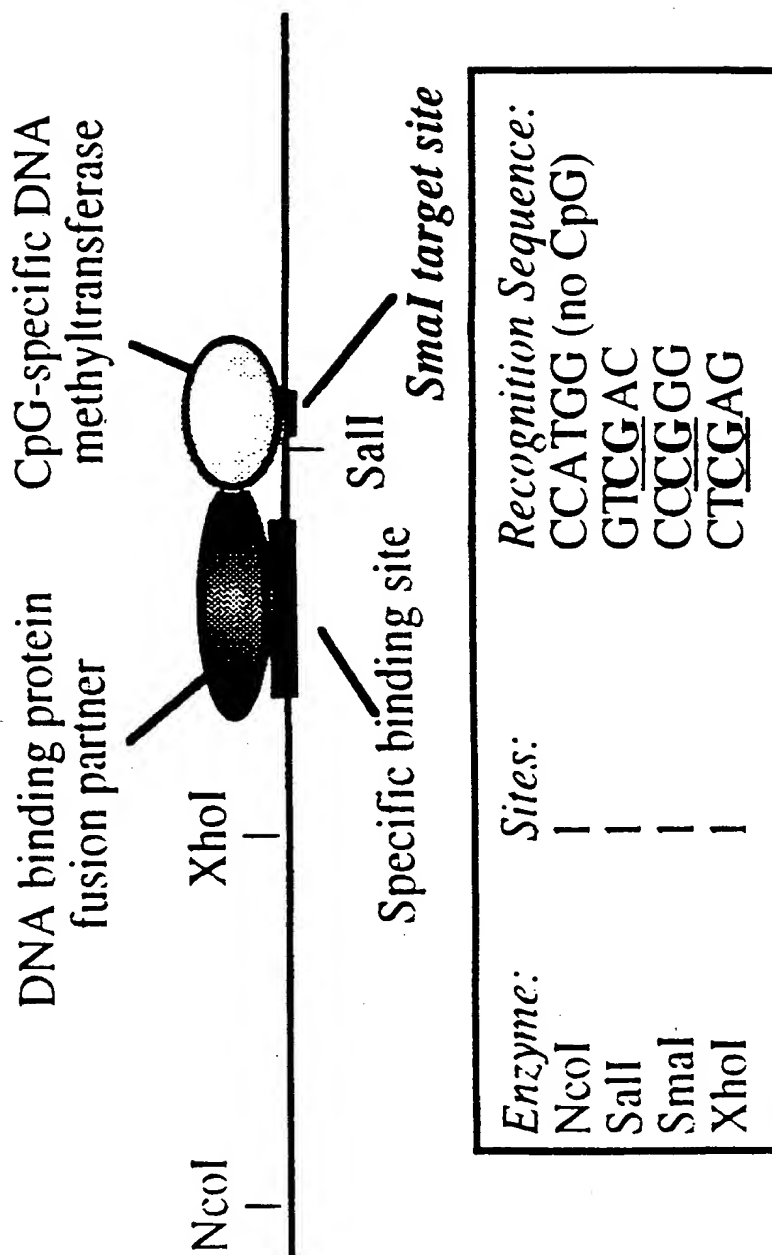pIII gene with 3 Cys_2-His_2
zinc finger modules

Phage fd-Tet-SN RF DNA

Random codons at contact positions

*Produce combinatorial library by
lytic growth in susceptible E. coli*

pIII coat protein   Phage fd-Tet-SN

zinc fingers

Avidin
paramagnetic
bead

Biotin—C_6TG GAGCCTGC GG T T T
            ACACTCGGACG CC T T T

*Elute bound phage with specific oligo
or 0.1 M triethylamine*

Determine specificity and affinity of selected
zinc finger proteins by mobility shift assay

FIGURE 10

CpG Sites and Viral Transcripts in Hepatitis B Virus
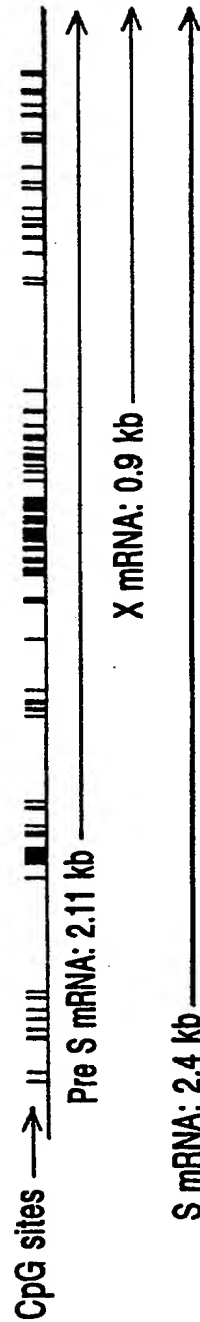
CpG sites

Pre S mRNA: 2.11 kb

S mRNA: 2.4 kb

X mRNA: 0.9 kb

09/051013

FIGURE 11A

PCT/US96/15576

12/18

FIGURE 11B

FIGURE 12

FIGURE 13

PBS   pLM9

←LacI-M.SssI Fusion
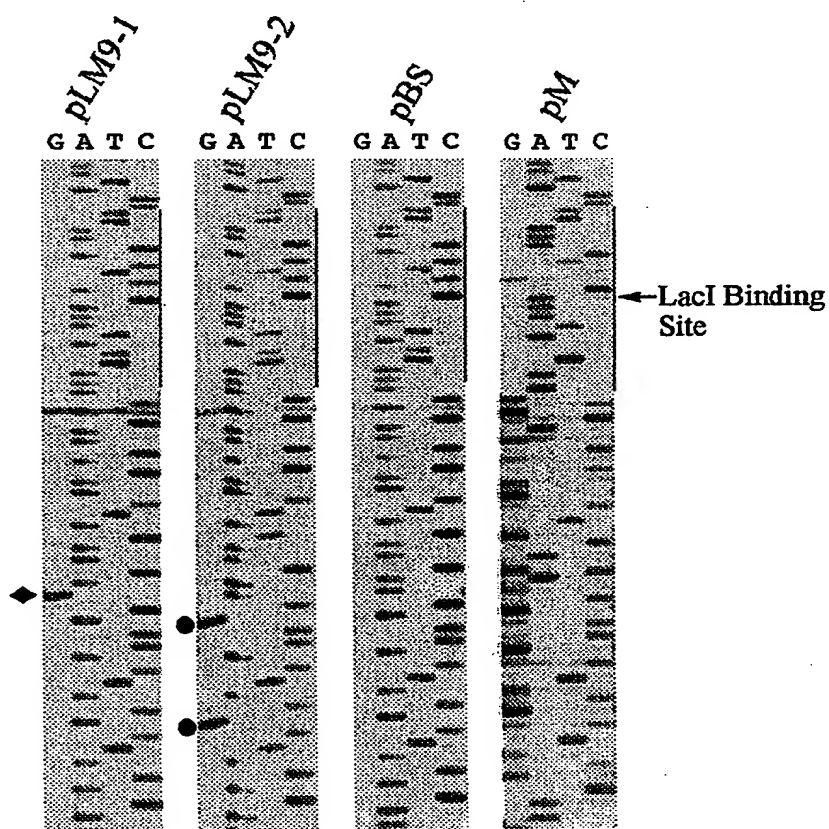(80 KD)
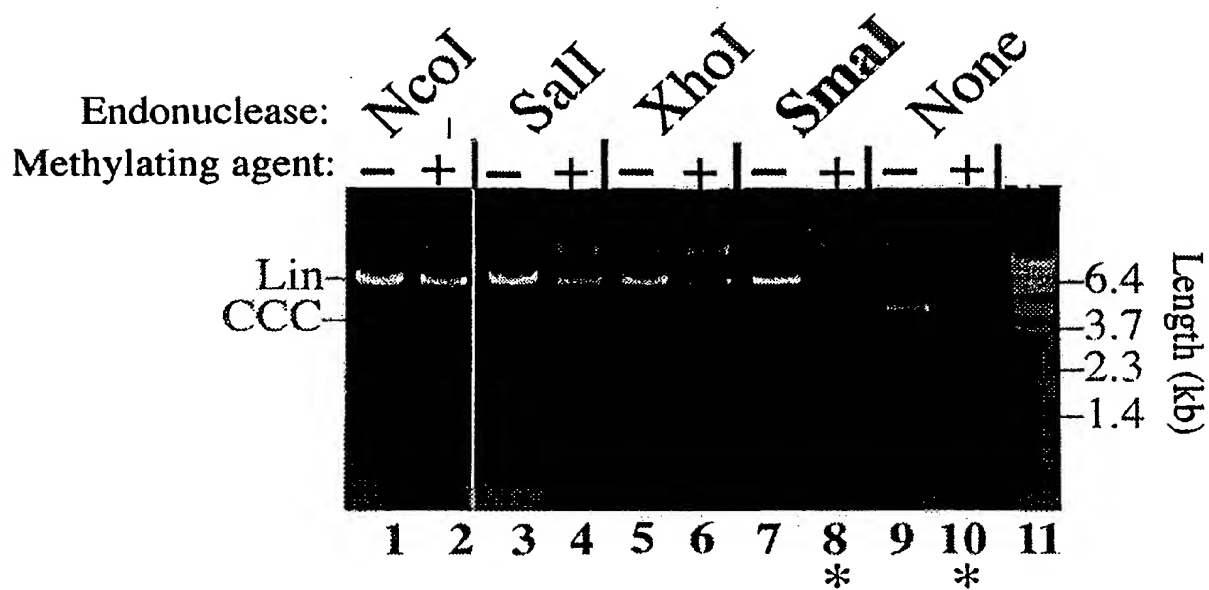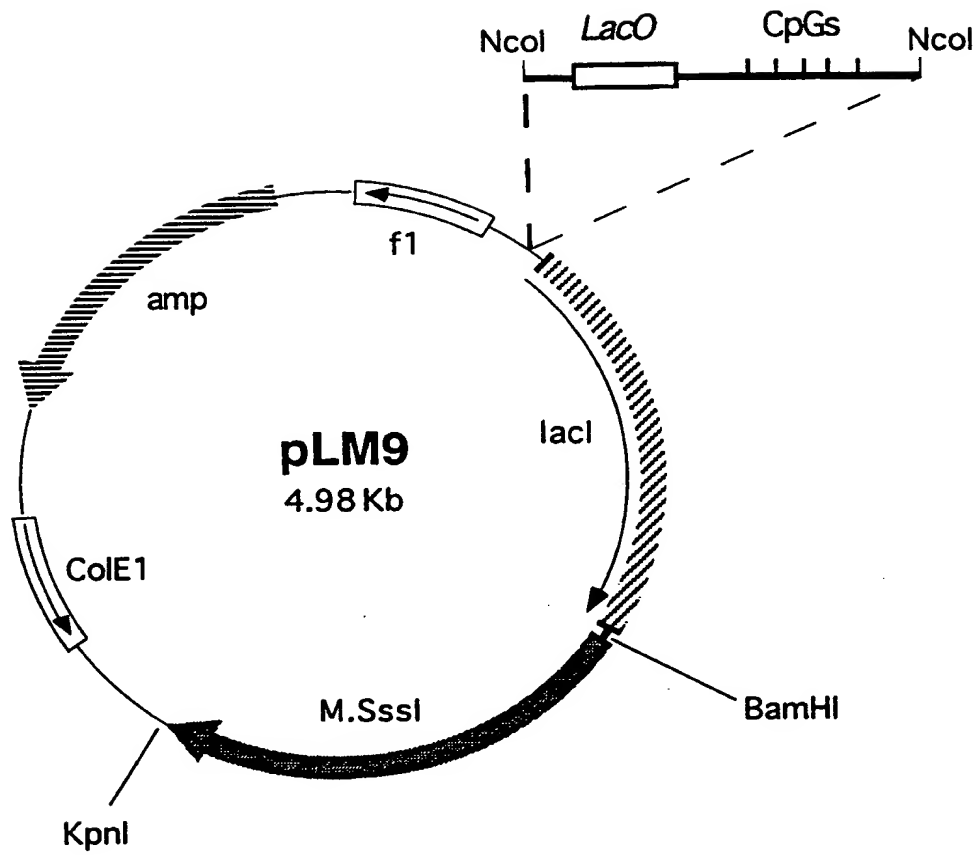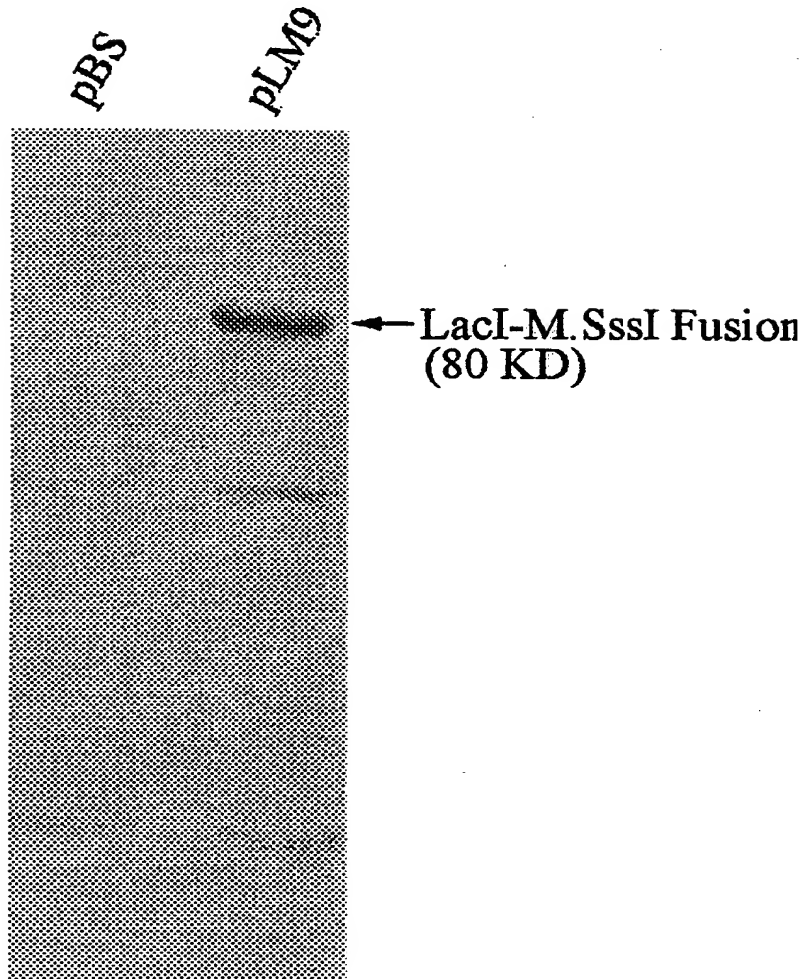
FIGURE 14

LacI Recognition Sequence

CCATGGAATTGTGAGCGCTCACAATTCGCGTCGCGCCGACGCCGTCGCGGCGACGCGACGCGTT
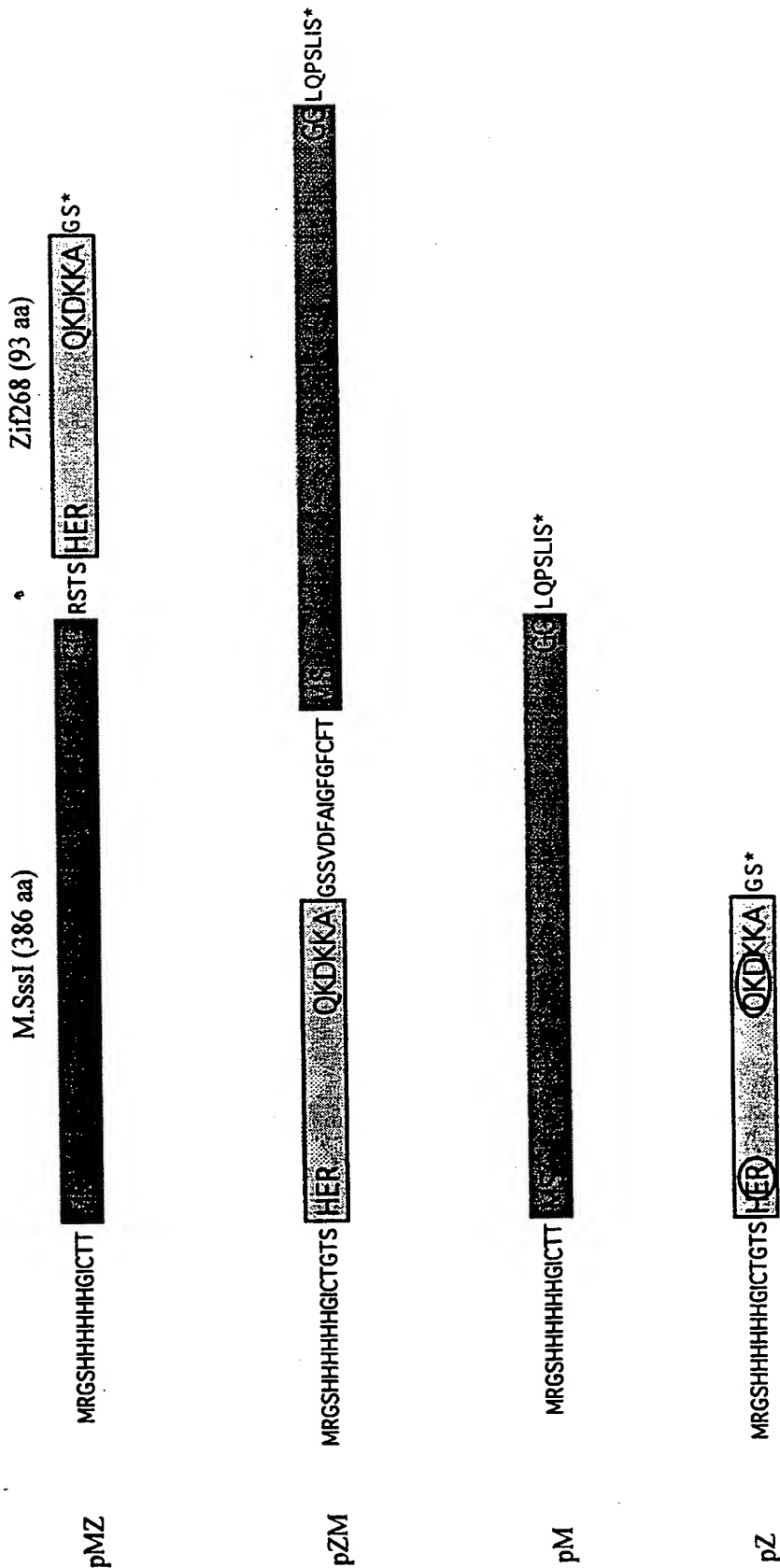GGTACCTTAACACTCGCGAGTGTTAAGCGCAGCGCGGCTGCGGCAGCGCCGCTGCGCTGCGCAA

pLM9-1   pLM9-2   pBS   pM
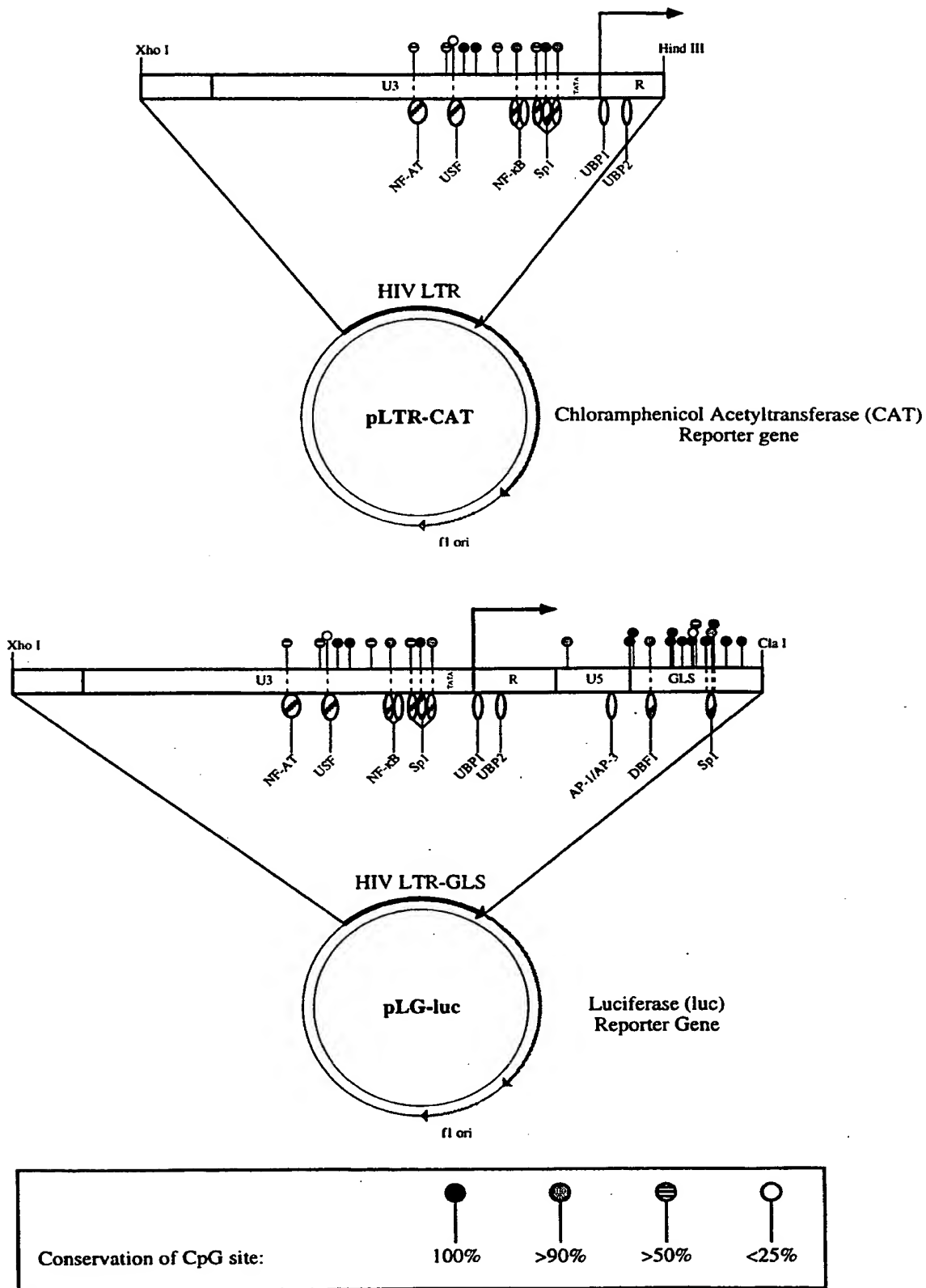
G A T C   G A T C   G A T C   G A T C

←LacI Binding Site

FIGURE 15

Constructs for the Expression of Zinc finger-DNA methyltransferase Fusion Proteins

FIGURE 16

pLTR-CAT

Chloramphenicol Acetyltransferase (CAT) Reporter gene

pLG-luc

Luciferase (luc) Reporter Gene

Conservation of CpG site: 100% >90% >50% <25%

FIGURE 17